# Identification and description of unknown *Brassica rapa* L. accessions kept in European Gene banks (in Bras-EDB) for the management of *Brassica* genetic resources

Organization: N.I.Vavilov Research Institute of Plant Industry (VIR)
Department of Vegetable crops and Cucurbits
Researcher: Dr. Anna M. Artemyeva

## Introduction

During the ECPGR Vegetable Network Meeting (Catania, November 2009) and the Brassica Working Group meeting (Catania, March 2010) it was decided to develop an ECPGR Phase VIII project and that this project should involve both wild *Brassica* species and *Brassica rapa*. For these species, there are a number of accessions held in the European collections that are poorly characterized.

This scope of work relates to *Brassica rapa*, which could be defined as a multicrop species for its multiple utilization. The main problem for *Brassica rapa* is that sometimes accessions have been included in Gene banks databases without correct botanical names and it is necessary to improve the existing *Brassica* database by filling these gaps and to have better information in selected AEGIS accessions. It was agreed to identify 'unknown' accessions of *Brassica rapa* with the aim of characterizing and evaluating them for several aspects such as bio-morphological traits, oils and nutraceutical compounds, DNA analysis, etc.

Criteria for selected *Brassica rapa* accessions for the project were 1. *B. rapa* accessions of which no information on the subtaxa are present in the Bras-EDB; 2. availability of seed samples to use; 3. several donor/origin countries.

The members of the Brassica WG are contributing to this initiative in relation to their main interest and competences. The activities are mainly cofinanced by specific projects which the members are carrying out and/or by the economical supports of breeders, research institutes/universities that are interested in regeneration, characterisation and evaluation activities. The results will also contribute to the implementation of AEGIS, since accessions will be proposed to be included as part of the European Collection. The characterization and evaluation activities will adopt common standards and descriptors.

The activities foreseen carried out by the Centre for Genetic Resources, the Netherlands (CGN), the Institute of Sustainable Agriculture of Cordoba (CSIC-Spain), and N.I.Vavilov Research Institute of Plant Industry (VIR). Antioxidant compounds are being analysed with common protocols, whereas for DNA molecular analysis, standard SSR primers have been used.

## Description of the work

- Identify the subtaxa, conduct morphological and biological description of 57 *B.rapa* accessions in the field in Saint-Petersburg area, north-Western Russia, in summer 2012, in good fertilized soil conditions.
- Conduct biochemical analysis on 57 accessions, including content of dry matter, proteins, sugars, ascorbic acid, carotenoids, carotenes, chlorophylls, organic acids, amino acids, fatty acids, phenol compounds, spirits, and others.
- Make DNA microsatellite analysis of 57 accessions using SSR markers covering all 10 *B.rapa* linkage groups.
- Add the obtained data to the Bras-EDB in order to make them publicly available.
- Write a technical report.

## Material and methods

57 "unknown" *Brassica rapa* L. accessions were obtained by VIR from IPK, Germany (36 acc.), CGN, Netherlands (11 acc.), Warwick, HRI, Great Britain (9 acc.), Inst. for PGR, K. Malkov, Bulgaria (1 acc.). 48 accessions originated from 16 countries: Italy (18 acc.), Pakistan (8), Portugal (3), China (3), France (2), Great Britain (2), Thailand (2), Cuba (1), Iraq (1), Russia (1), Uzbekistan (1), Netherlands (1), Ireland (1), Columbia (1), Georgia (1), Malaysia (1), of 9 accessions the origin was unknown.

The 57 accessions have been grown in the field in Saint-Petersburg area, North-Western Russia, in summer 2012, in good fertilized soil conditions, and in glass greenhouse. The date of sowing in the field was 2nd July, in the greenhouse – 3rd May. For each accession twenty plants were planted out in the field and ten in the greenhouse.

Morphological and biological description has been done according VIR descriptors accepted by VIR after the works of E.N.Sinskaya and T.V.Lizgunova that included 64 characters: 1 phenological trait, 6 growth-related traits, 19 leaf traits, 8 head traits, 8 root traits, 9 flower traits, 9 pod traits, 4 seed traits (Annex 1).

DNA microsatellite analysis has been done in the VIR laboratory of Molecular-Ecological Genetics using 10 SSR markers covering all 10 *B.rapa* linkage groups according to a standard protocol (Annex 2).

*SSR (Simple Sequence Repeats) DNA analysis*

We used 10 oligonucleotide primers pairs in the investigations of the *Brassica* species. The primers BC7, BC48, BC51, BC63, BC65, BC105, BC107 are described by Ma Rongcai (unpublished data), Ra2E12 by Lowe et al (2004), BRMS019 and BRMS050 by Suwabe et al (2006). DNA was extracted from young leaves as described by Dorokhov and Klocke (1997). For heterogenic bulk analysis 5 plants per each accession were used.

PCR was carried out in a mixture with a volume of 12.5µl: 10×incubation buffer (1,25 µl), 2.5 mM $MgCl_2$, 0,25 µl of each dNTP (10 mM), of 0,25 µl each primer (10 pmol/ µl), 0,1 µl Taq DNA polymerase (5 U/ µl), and 20 ng genomic DNA. Amplification was carried out in a DNA thermocycler (Biorad, Germany) programmed for BC primers for 43 cycles: cycle 1, primary denaturation at 94°C for 3 min; cycle 2, at 94°C for 1 min; annealing of primer sat using touch-down from 65°C till 56° for 1 min. The temperature was lowered 1°C each cycle until reaching an annealing temperature of 56°C. Extension was carried out at 72°C for 45s (20 cycles). Cycle 4 was carried out at 94°C for 1 min, annealing at 55°C for 1 min (30 cycles), and extension at 72°C for 5 min. Final temperature was 4 ºC. The amplification products were separated by electrophoresis in 2.5% agarose gel in 0.5×TBE buffer, stained in ethidium bromide, and documented.

SSR binary matrix was constructed by using (1) for the presence and (0) for absence of the marker,
the similarity indexes were calculated with the use of genetic distance according to Nei and Li (1979). Cluster analysis was performed by the UPGMA method by TREECON for Windows (version 1.36). The linkage distances were calculated per 100 units and expressed in percent.

Biochemical analysis of leaves, roots, heads including content of dry matter, proteins, sugars, organic acids, ascorbic acid, carotenoids, carotenes, chlorophylls, and biochemical analysis by GLC method was conducted in the VIR laboratory of Biochemistry by standard methods.

*Extraction*

For determination of ingredients 1 g of the fresh material was used. Then samples were boiled in glass-tube for 1 min with 80% Ethanol, grinded and filtered. The filtrate was

used for GLC method and for analysis 0,4 ml filtrate was used and evaporated. After in samples were add 20 µl internal standard Tricosan (nC23) and 50 µl silylate agent, then heated for 1 hour at 100°C and put into a vial for the GLC-analysis.

*GLC-method*

Detector: Agilent

Programme oven: Initial temp: 70 C (On)
Maximum temp: 325 C
Equilibration time: 0.50 min

Ramps:

| # | Rate | Final temp | Final time |
|---|---|---|---|
| | 6.00 | 320 | 20.00 |

Flow: Saver flow: 15.0 mL/min
Saver time: 2.00 min
Gas type: Helium

Time: 61.67 min

Column: SIGNAL
Model Number: 19091S-433E
Description: HP-5MS 5% Phenyl Methyl
Max temperature: 325 C
Nominal length: 30.0 m
Nominal diameter: 250.00 um
Nominal film thickness: 0.25 um
Mode: constant flow
Initial flow: 1.3 mL/min
Nominal init pressure: 0.832 bar
Average velocity: 42 cm/sec

MS parameters: Solvent Delay : 4.00 min
EMV Mode : Relative
Relative Voltage : 0
Resulting EM Voltage : 1612

The leaves of all accessions were dried in Freeze-dryer and sent for glucosinolates analysis to Spain to Institute of Sustainable Agriculture – CSIC, Department of Plant Breeding, Prof. Antonio de Haro-Bailón.

**Results**

All *B. rapa* subspecies were identified among selected accessions: broccoletto *B.rapa* ruvo group (10 pure accessions and 3 acc. mix with broccoli *B.oleracea botrytis italica* and *B.rapa purpuraria*), turnip *B.rapa rapa* (pure turnip 7 acc. and 4 acc. mix with *B.napus* and others *brassicas* and 1 acc. under question or synthetic turnip), leafy turnip *B.rapa rapa komatsuna* type (3 acc.), pak-choi *B.rapa chinensis* (1 acc.), choy sum *B.rapa parachinensis* (3 pure acc. and 1 acc. mix with *B.napus* and *B.juncea*), honsaitai *B.rapa purpuraria* (1) acc.), Chinese cabbage *B.rapa pekinensis* (2 acc.), oilseed rape *B.rapa oleifera* (4 pure acc. and 2 acc. mix with broccoletto and *B.napus*), *B.rapa sylvestris* ? (3 acc.), and also *B.napus* (3 acc.) and *B.juncea* (9 acc.).

All accessions were described using 64 characters (attached file). The photos are presented in attached file.

52 polymorphic bands were found that provided better knowledge on the diversity of this set (see Annex 3).

43 acc. have been evaluated by GLC (attached file).

## Conclusions

57 "unknown" *Brassica* accessions have been studied and characterised according to 64 characters. All *B. rapa* subspecies were identified among selected accessions. 3 accessions were identified as *B. napus*, 9 as *B. juncea*. For the *B. rapa* accessions the crop types were identified which included broccoletto, turnip, leafy turnip, pak-choi, Chinese cabbage, choy sum, honsaitai, oil seed rape, but also mixtures have been observed.

DNA microsatellite analysis using SSR 10 markers covering all 10 *B.rapa* linkage groups has been done, 52 polymorphic bands were found that provided better knowledge on the diversity of this set. In our study the clusters on dendrogram do not correspond well to botanical division, possibly as results of two causes: 1) we used 7 primer pairs from Ma Rongcai what we have used before for analysis of VIR *Brassica rapa* collection and the clusters of Chinese cabbage, pak-choi, tatsoi, mizuna and oilseed were correct, whereas the accessions of turnip were dispersed between other accessions mostly in cluster with oilseed, possibly because of high level of their genetic variability. We have not analyzed accessions of broccoletto totally in our previous works. So it seems that part of primers that we used now more convenient for analysis of Asian vegetables and not so good for analysis of turnip and broccoletto. 2) We used now electrophoresis in agarose gel which gives not so clear and accurate positions of the bands as PAA gel and moreover LiCor or similar equipment.

Additionally, biochemical analysis of leaves, roots, heads, including content of dry matter, proteins, sugars, ascorbic acid, carotenoids, carotenes, chlorophylls, organic acids, amino acids, fatty acids, phenol compounds, spirits, and others compounds was conducted; valuable sources of high level of ascorbic acid and carotene were found.

Obtained results from this characterisation and analysis will be made publicly available via the website of BrasEDB. 26 *B.rapa* accessions have been proposed to be included in AEGIS (Annex 4).

The work here described has been approved by the Brassica Working Group and by the Coordinators of the Vegetable Network for the use of these funds.

## References


Dorokhov DB, Klocke E. (1997). A rapid and economic technique for RAPD analysis of plant genomes. Russ. J. Gen. 33: 358-365.

Lowe et al. (2004). Efficient large-scale development of microsatellites for marker and mapping applications in Brassica crop species. Theor. Appl. Genet. 108, 1103-1112, 10.1007/s00122-003-1522-7.

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleasis. Proceedings of the National Academy of Sciences of the USA, 76, 5269 – 5273.

Suwabe et al (2006) Simple sequence repeat-based comparative genomics between Brassica rapa and Arabidopsis thaliana: The genetic origin of clubroot resistance. Genetics. (173) 309-319.

**Annex 1**

**List of *Brassica rapa* analyzed traits**

| Trait type | Trait name | Trait description | Scale |
|---|---|---|---|
| Flowering time | Flowering time | Days from germination to appearance of the first open flower | days |
| Growth-related traits | Plant diameter | | cm |
| | Plant height | Height from ground to the leafs top | cm |
| | Leaves position | Leaves are horizontal-1, weekly uprisen-3, uprisen-5, strong uprisen-7, go up-9 | 1-9 |
| | Plant habit | Compact-1, semi-spreading-5, spreading-9 | 1-9 |
| | Leaves weight | | g |
| | Head/root weight | | g |
| Leaf traits | Leaf type | Entire sitting-1, indistinct lyre-3, lyre-5, entire with petiole-7 | 1-5 |
| | Petiole length | Length from base of petiole to bottom of lamina | cm |
| | Petiole width | Width of the petiole base | cm |
| | Petiole thickness | Thickness of the petiole base | cm |
| | Number of primary lobes | Absent-0, present-number | number: 1-3 |
| | Number of secondary lobes | Absent-0, present-number | number: 1-5 |
| | Petiole colour | White-1, light-green-5, green-9 | 1-9 |
| | Petiole surface | Flat-1, faint concave-3, concave-5, strong concave-7, convex-9 | 1-9 |
| | Petiole border | Absent-1, border on the base of petiole-3, border till the middle of petiole-5, border on almost all petiole-7, border on full petiole-9 | 1-9 |
| | Lamina length | Length from bottom to top of lamina | cm |
| | Lamina width | Lamina width at the widest point | cm |
| | Lamina shape | Ovate-1, obovoid-2, long oval-3, long ellipse-4, oval-5, truncated oval-6, ellipse-7, truncated ellipse-8, round-9 | 1-9 |
| | Lamina surface | Flat-1, faint concave-3, strong concave-5, faint convex-7, convex-8, strong convex-9 | 1-9 |
| | Surface of lamina tissue | Smooth-1, very weak rugate-2, weak rugate-3, middle rugate-4, strong rugate-5, plicate-rugate-6, minute bullate-7, middle bullate-8, strong bullate-9 | 1-9 |
| | Lamina nervation | Rare-1, middle-5, dense-9 | 1-9 |
| | Lamina edge | Smooth-1, very faint undulate-3, faint undulate-5, undulate-7, strong undulate-9 | 1-9 |

| | | | |
|---|---|---|---|
| | Cuttingness of edge | Absent-1, indistinct dentate-3, very minute dentate-5, minute dentate-7, dentate-9 | 1-9 |
| | Lamina colour | Light green-1, light grey green-2, bright light green-3, grey green-4, green-5, bright green-6, dark green-7, bright dark green-8, dark grey green-9 | 1-9 |
| | Hairiness | Absent-1, very weak on edge-3, weak-5, middle-7, strong-9 | 1-9 |
| Head traits | Head height | Length from bottom to top of head | cm |
| | Head diameter | Diameter at the widest point | cm |
| | Head shape | Flat-1, round-2, round-flat-3, oval-4, short oval-5, long oval-6, cylindrical-7, large cylindrical-8, long cylindrical-9 | 1-9 |
| | Head exterior colour | Light green-1, green-5, dark green-9 | 1-9 |
| | Head interior colour | White-1, white yellow-3, white green-5, yellow-7, yellow green-9 | 1-9 |
| | Head thickness | Crumbly-1, middle-3, thick-5, very thick-7 | 1-7 |
| | Core length | | cm |
| | Core width | | cm |
| Root traits | Root height | | cm |
| | Root diameter | | cm |
| | Root top diameter | | cm |
| | Root shape | Bulging-1, cylindrical-2, conic-3, large conic-4, long conic-5, oval-6, round-7, round flat-8, flat-9 | 1-9 |
| | Root top skin colour | White-1, yellow-3, green-5, purple-7, grey-9 | 1-9 |
| | Root bottom skin colour | White-1, yellow-3, green-5, purple-7, grey-9 | 1-9 |
| | Root flash colour | White-1, yellow-5, green-9 | 1-9 |
| | Root skin surface | Smooth-1, ribbed-3, week ribbed-5, strong ribbed-7 | 1-7 |
| Flower traits | Flower diameter | | cm |
| | Flower height | | cm |
| | Length of pedicle | | cm |
| | Shape of petal tip | Obtuse-1, obtuse with groove-3, faint pointed-5, pointed-7 | 1-7 |
| | Surface of petal | Smooth-1, very weak rugate-3, weak rugate-5, middle rugate-7, strong rugate-9 | 1-9 |
| | Flower colour | Pale yellow-1, light yellow-3, yellow-5, bright yellow-7 | 1-7 |
| | Bend length | | cm |
| | Bend width | | cm |
| | Claw length | | cm |

| | | | |
|---|---|---|---|
| Pod traits | Pod length | | cm |
| | Pod width | | cm |
| | Pod shape | Flat-1, flattened-cylindrical-3, flattened-5, strong flattened-7, cylindrical-9 | 1-9 |
| | Pod surface | Smooth-1, weak hillocked-3, hillocked-5, strong hillocked-7 | 1-7 |
| | Beak length | | cm |
| | Beak width | | cm |
| | Beak shape | Sharp-1, faint pointed-3, pointed-5, obtuse-9 | 1-9 |
| | Deflection from stem | | ° |
| | Pod colour | Pale green-1, light green-3, green-5, bright green-7, dark green-9 | 1-9 |
| Seed traits | Seed diameter | | mm |
| | Seed shape | Round-1, round-flat-3, oval-5, angular-7, irregular-9 | 1-9 |
| | Seed surface | Smooth-1, rugate-9 | 1-9 |
| | Seed colour | Brown-1, light-brown-2, dark-brown-3, yellow-4, light-yellow-5, dark-yellow-6, green-7, green-brown-8, yellow-green-9 | 1-9 |
| Biochemical traits | Dry matter | | mg/100g |
| | Total sugars | | mg/100g |
| | Monosugars | | mg/100g |
| | Protein | | mg/100g |
| | Ascorbic acid | | mg/100g |
| | Chlorophyll a | | mg/100g |
| | Chlorophyll b | | mg/100g |
| | Carotenoids | | mg/100g |
| | Carotens | | mg/100g |
| | Carotene α | | mg/100g |
| | Carotene β | | mg/100g |

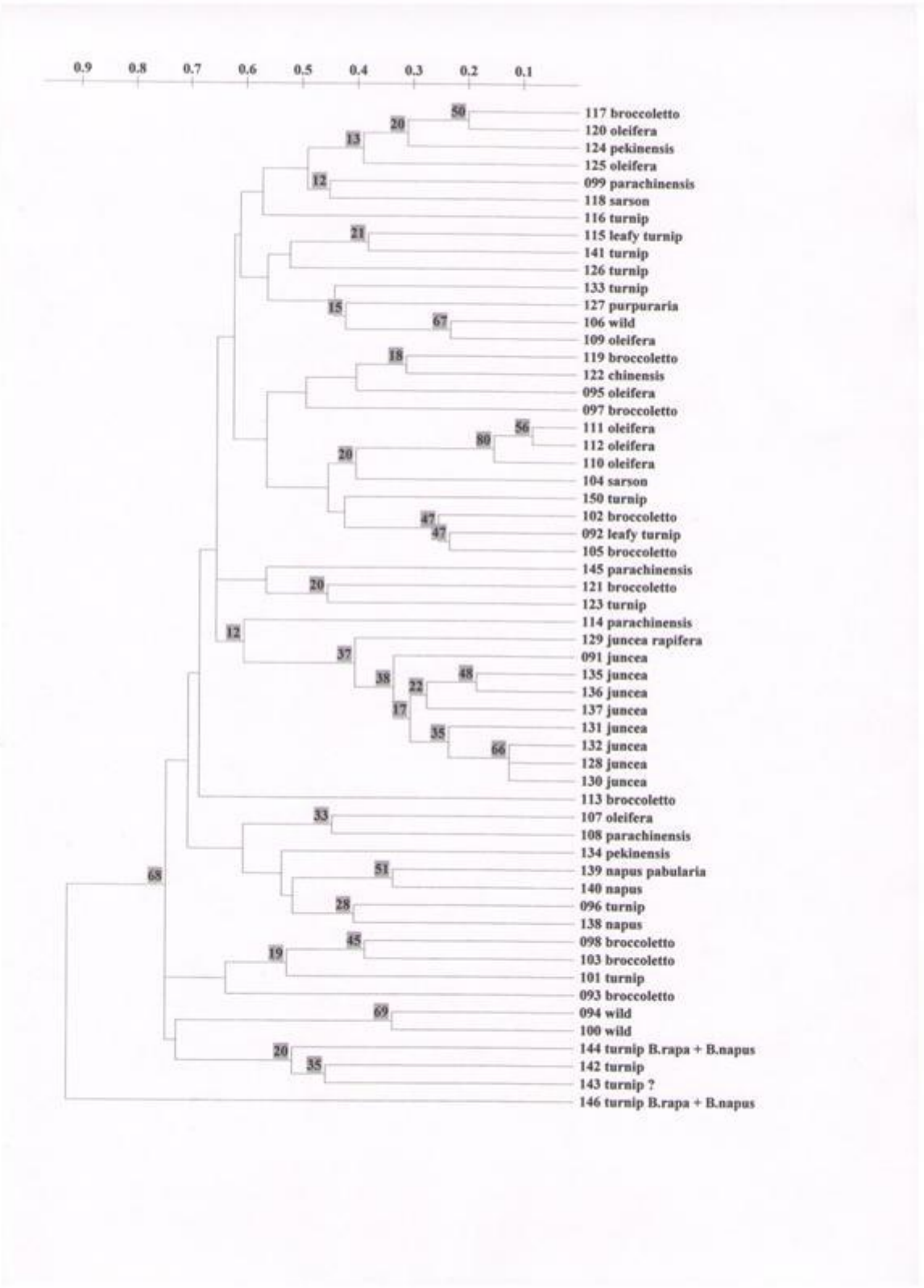
**Annex 2**

SSR markers used in project

| Locus name/primer name | Motif | Sequences (forward, reverse) | Linkage group | Observed size of amplicons (bp) | Number of alleles |
|---|---|---|---|---|---|
| BC7 | $(ACC)_6$ | AGTTGGCCCCATTTCATTGTTAT<br>CATCTTGACGGCCTCCATCTCCA | A01 | 150-310 | 8 |
| BC48 | $(TCT)_7$ | GGTGGTGGGCTGGGGAGTA<br>CGTCGATCGATTCATAACCGTAGA | A02 | 230 – 320 | 5 |
| BC51 | $(GAA)_6$ | CCGAGGAAGAAAGCTGTTGAGTTG<br>ATCGCTTCCGTAGACACCTTCGTT | A06 | 146 – 180 | 4 |
| BC63 | $(AG)_9$ | TTCCGTCCCTTCCCTAAACA<br>GAACACTACTGCCCAGAGAACAC | A03 | 193-215 | 5 |
| BC65 | $(AG)_7$ | TTCCGTCCCTTCCCTAAACAA<br>TGAACACTACTGCCCAGAGAACAC | A04 | 190-215 | 4 |
| BC105 | $(CT)_7$ | GACGCCTCAATTGCTTACTT<br>AGGGAATGAGGATGGGTCTG | A05 | 207 – 214 | 3 |
| BC107 | $(TC)_{10}$ | ATACAATCTTCGTGACTCTACAG<br>AGCATCAACGCCAACTTTATCC | A09 | 284 – 295 | 3 |
| BRMS019 | $(GT)_{10}$ | CCCAAACGCTTTTGACACAT<br>GGCACAATCCACTCAGCTTT | A10 | 205-280 | 5 |
| BRMS040 | $(GA)_{49}$ $(GT)_4$ | TCGGATTTGCATGTTCCTGACT<br>CCGATACACAACCAGCCAACTC | A07 | 200-560 | 7 |
| Ra2E12 | | TGTCAGTGTGTCCACTTCGC<br>AAGAGAAACCCAATAAAGTAGAACC | A08 | 130-260 | 8 |

**Annex 3**

UPGMA dendrogram constructed by Nei& Li genetic distance calculation



**Annex 4**

*Brassica rapa* accessions for AEGIS

| INSTCODE | ACCENUMB | GENUS | SPECIES | ORIGCTY | Taxonomic determination at VIR-2012 |
|---|---|---|---|---|---|
| DEU146 | BRA 1778 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | BRA 2777 | *Brassica* | *rapa* | | Turnip *B.rapa rapa* |
| DEU146 | BRA 2780 | *Brassica* | *rapa* | | Turnip *B.rapa rapa* |
| DEU146 | BRA 2790 | *Brassica* | *rapa* | THA | Choy sum *B.rapa parachinensis* |
| DEU146 | BRA 2803 | *Brassica* | *rapa* | | Leafy turnip Komatsuna type *B.rapa rapa* |
| DEU146 | BRA 2810 | *Brassica* | *rapa* | CHN | Chinese cabbage *B.rapa pekinensis* Da-tsin-kou type |
| DEU146 | BRA 2812 | *Brassica* | *rapa* | | Leafy turnip Komatsuna type *B.rapa rapa* |
| DEU146 | BRA 2837 | *Brassica* | *rapa* | ITA | Turnip *B.rapa rapa* |
| DEU146 | BRA 2842 | *Brassica* | *rapa* | IRQ | Turnip *B.rapa rapa* |
| NLD037 | CGN06867 | *Brassica* | *rapa* | SUN | Chinese cabbage *B.rapa pekinensis* Granat type |
| NLD037 | CGN20196 | *Brassica* | *rapa* | UZB | Turnip *B.rapa rapa* |
| GBR006 | HRIGRU 12377 | *Brassica* | *rapa* | PRT | Turnip *B.rapa rapa* |
| GBR006 | HRIGRU 2489 | *Brassica* | *rapa?* | MYS | Choy sum *B.rapa parachinensis* |
| GBR006 | HRIGRU 7687 | *Brassica* | *rapa?* | | Turnip *B.rapa rapa* |
| DEU146 | K 6465 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 7858 | *Brassica* | *rapa* | ITA | Oilseed *B.rapa oleifera annual* |
| DEU146 | K 7866 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 7877 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 7884 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 7904 | *Brassica* | *rapa* | COL | Brown sarson *B.rapa dichotoma* |
| DEU146 | K 8083 | | | | Oilseed *B.rapa oleifera annual* |
| DEU146 | K 8471 | *Brassica* | *rapa* | THA | Choy sum *B.rapa parachinensis* |
| DEU146 | K 8642 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 9013 | *Brassica* | *rapa* | ITA | Broccoletto *B.rapa ruvo* |
| DEU146 | K 9394 | *Brassica* | *rapa* | | Leafy turnip *B.rapa rapa* |
| DEU146 | K 9708 | *Brassica* | *rapa* | CHN | Honsaitai *B.rapa purpuraria* |